SOME PECULIAR FERN PROTHALLIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 137

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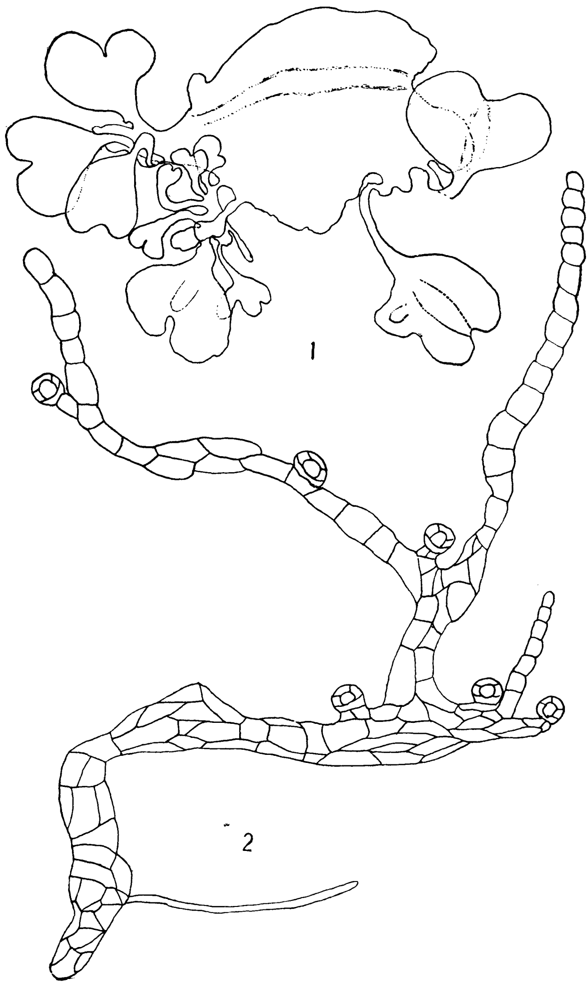
(WITH ELEVEN FIGURES)

In September 1906 Miss S. M. HAGUE sent me some fern prothallia from a swamp in northern Indiana, where they were growing luxuriantly on rotten wood. Pieces of the wood were put into a glass jar, which was covered and placed on a table about seven feet from a window with a southeast exposure. They were kept very moist, yet watered with care, so as not to allow any water to get on the plants and thus cause fertilization. In spite of this precaution, however, enough moisture probably collected on the plants to permit fertilization occasionally, for sporophytes developed at intervals. Some of these, as well as many of the gametophytes, were used in class work. A few of the sporophytes are about 7 cm. high. They resemble SLOSSON'S figures of *Dryopteris spinulosa intermedia* (*Aspidium intermedium* Muhl.). Certain peculiarities found in this material seem worth describing. Some of the gametophytes are still growing and will be watched for further developments.

Gametophytes

The prothallia were of the typical heart-shaped form, the larger ones being about 5 mm. long. Those on which sporophytes have not developed have continued to grow, and many spores, which had been lying for a long time in the rotton wood, have germinated. The old prothallia have grown to unusual size and taken queer shapes, many of them being 15 mm. long, a few 23 mm., and one even measuring 37 mm. The most striking thing about them, however, is the peculiar forms developed. A hasty observation of one of these often gives the impression of a number of prothallia near together and overlapping, for they often branch, apparently from any part of the plant, as in fig. 1, which is a diagram of one of the simpler cases. At one point near the margin of another plant 21 of these branches were counted, all 1–1.5 mm. long, and each bearing 6–10 antheridia. In

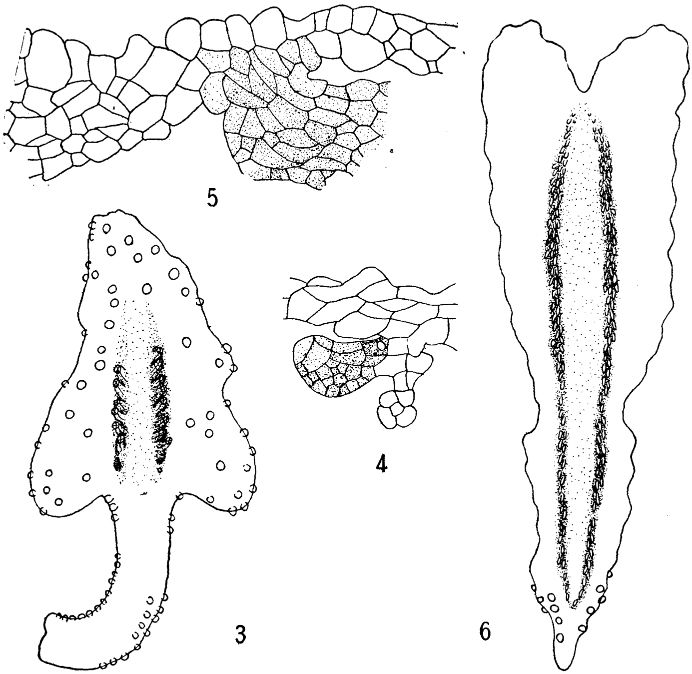
some cases filamentous processes grow out (fig. 2); this figure was made from fresh material, and the cell walls may not be accurate in



FIGS. 1, 2.—Fig. 1, diagram of one of the simpler prothallia with several gametophytic branches; fig. 2, sketch of a filamentous process with antheridia, from living material.

every case. Occasionally a cylindrical process was seen, as described by LANG (2), but it might possibly have developed into the ordinary form, for some of the branches have slender bases. In several

instances this outgrowth is from the apical region, as shown in the diagram (fig. 3), which represents a small prothallium 5 mm. long. The outgrowth has antheridia along the margins, but shows no archegonia; these were present, however, on the main prothallium.



FIGS. 3–6.—Fig. 3, prothallium 5 mm. long, with apical process bearing antheridia but no archegonia, which are found on the main prothallium; fig. 4, section through a very young gametophytic branch with apical cell; the branch is at the edge of the section and near an archegonium, the cross-section of the neck of which is shown; fig. 5, section through the basal part of a gametophytic branch, showing its relation to the main gametophyte; fig. 6, an antheridial prothallium 23 mm. long, with many archegonia but no antheridia except in the basal region.

Sections of this prothallium show nothing unusual in structure or cell contents.

A section of one of these branches at a very early stage shows its relation to the main prothallium (fig. 4). It comes from the edge of the archegonial cushion, the section showing also the neck of an adjacent archegonium. So far as the apical region is concerned, it

looks like a normal gametophyte. The basal part of a somewhat older branch is shown in fig. 5, which is broader and does not seem so closely related to archegonia, although near the cushion. Apparently any cell or group of cells may be rejuvenated and initiate this branching. The cells in the neighborhood of fig. 4 are very large for cells in a position so near the apical region, and they contain unusually large food bodies, apparently oil globules; this may be a condition that leads to branching.

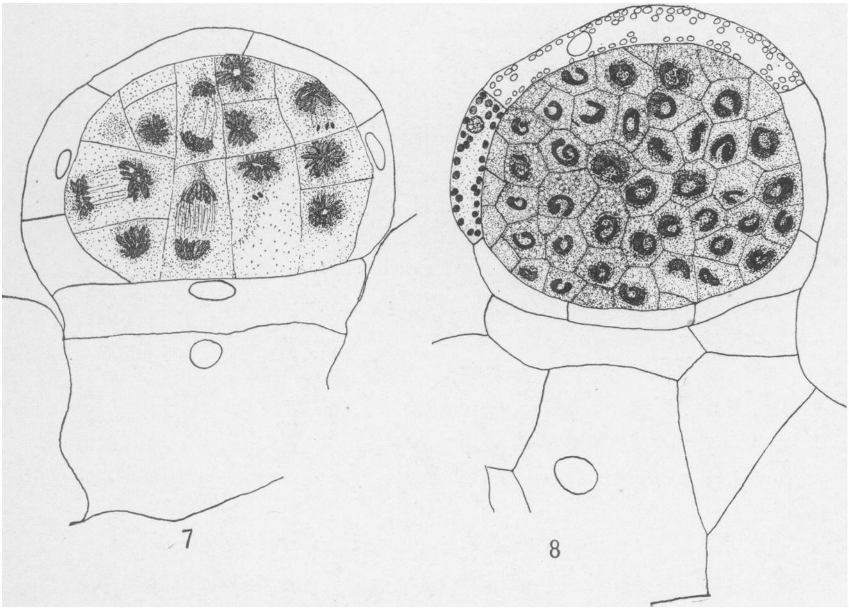
Another type of gametophyte is shown in fig. 6. This prothallium does not branch and is 23 mm. long. There are few or no antheridia on prothallia of this type except near the basal region, but the archegonial cushion is unusually wide and thick, and has a broad row of archegonia along both margins, with more in the center. Transversely, these rows have 1–5 archegonia very closely crowded together. In one gametophyte 16 mm. long, there were found by actual count 142 archegonia on one side of the cushion, with apparently as many on the other side; so that instead of the usual small number of archegonia, there were probably not far from 300 on this particular gametophyte, and other gametophytes of this type. The production of archegonia apparently goes on indefinitely, for the prothallia still looked vigorous.

Antheridia and archegonia

A rather complete series of stages in the development of antheridia and archegonia was examined, but most of them resembled the usual type. Two late antheridial stages are shown in figs. 7 and 8, the former with the spermatogenous cells in mitosis, and the latter with sperms almost mature. There are apparently 16 chromosomes, though none of the figures are in condition to permit absolute accuracy in counting. As a rule, antheridia are found only on the basal region of old prothallia or on the branches, but occasionally one or two appear at any point on the gametophyte. In a few instances immature antheridia were found among mature archegonia. An unexpected feature in archegonial development is their appearance far back from the growing point, so that young archegonia are found among the old ones. A peculiarity several times noted was two archegonia with no wall between the egg cells, but with two complete necks; otherwise nothing unusual was seen in their development.

Fertilization

A few prothallia were placed in water for a short time, and fertilization took place in the usual way, one or more sperms entering the neck and reaching the so-called receptive spot, where one sperm enters the egg. The sperm nucleus fuses slowly with the egg nucleus,



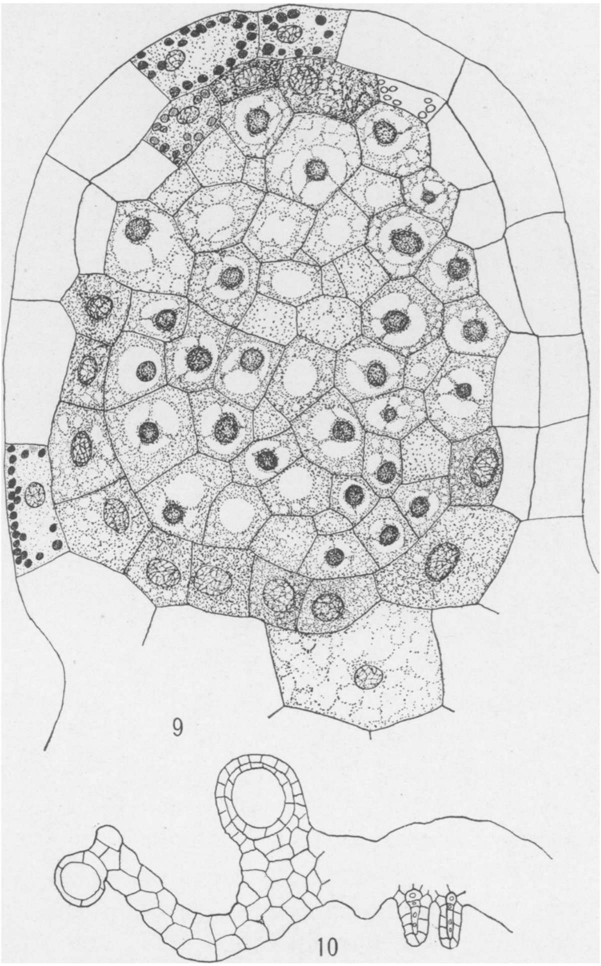
FIGS. 7, 8.—Fig. 7, antheridium with spermatogenous cells in mitosis, showing approximately 16 chromosomes; fig. 8, antheridium with sperms almost mature.

the fusion apparently being completed while both are in the resting condition. A few normal embryos in well-advanced stages were found. These must have been formed from the fertilized egg or have been developed parthenogenetically.

Apogamy

A peculiar structure was found on the gametophyte which bore the antheridia shown in fig. 8, but it was found on the upper surface. The diagram (fig. 10) shows the relation of this body and the antheridium to each other and to the archegonia. It is clear that there are only two possible interpretations of this structure: it must be an

abnormal antheridium, or it is a sporangium; if it is the latter, this is a case of apogamy. The great difference in size is seen by com-



FIGS. 9, 10.—Fig. 9, a sporangium-like structure from the upper surface of the gametophyte from which was drawn fig. 8; two layers of cells outside the fertile region, the inner layer suggesting a tapetum; each layer consists of many cells; fig. 10, diagram showing the relation of figs. 8 and 6 to one another and to the archegonial region.

paring the figures, which are drawn to the same scale. Among all the antheridia examined from this material, there is no such difference in size, the antheridium shown in fig. 8 being a rather large one. In

all the antheridia only one layer of wall cells is present, and these are few and rather definitely placed. But this structure shows two distinct layers (figs. 9, 10), except in one section, and this at only one place (fig. 11). Here for the space of two cells the wall is only one cell thick, but these two cells might divide and make the two layers complete. The outer layer consists of many cells, which always contain chloroplasts, but the next layer contains chloroplasts in a few cells only. The nuclei in this second layer are quite different from those in the interior, and in some of the cells there is a suggestion of the usual tapetal appearance. It does not have the long slender stalk characteristic of the normal sporangia of the higher Polypodiaceae, but it probably would have elongated somewhat before maturity, making it very similar to the stalks of the Osmundaceae. Consequently, this structure is quite unlike an antheridium, and is almost perfect in its sporangial characters. As there were no mitotic figures, it could not be determined whether the nuclei contained the sporophytic or gametophytic number of chromosomes, but we should expect the sporophytic number to appear, especially since another prothallium bore a mature sporangium of the *Osmunda*, type, with a few spores still remaining in the sporangium. In the absence of mitotic figures, such a sporangium easily invites speculation. It

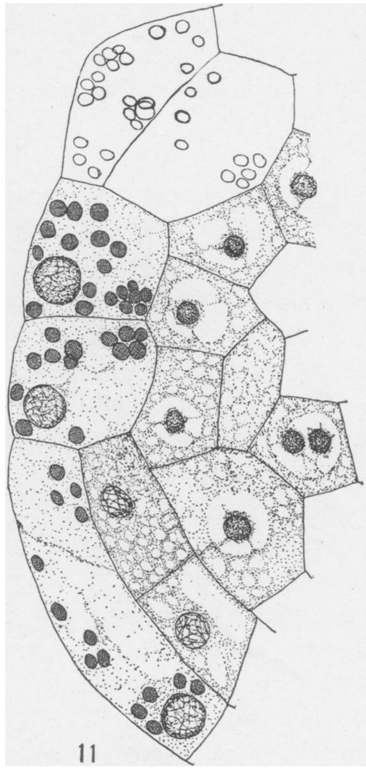


FIG. 11.—A small portion of the same sporangium-like structure shown in fig. 9, but from another section; this is the only place where one layer of cells appears outside the fertile region, and these two cells might divide and make the two layers complete.

may be an x structure, and the spores may have been formed in the usual way, so that the new gametophyte will have only $\frac{1}{2}x$ chromosomes; an interesting surmise in view of the fact that nearly related forms are known to show 12, 24, and 48 chromosomes. Or there may have been a doubling of the number and a subsequent reduction at the formation of spores. The appearance of a sporangium upon a prothallium will be accepted as a case of apogamy.

Discussion

LANG (**2**), in his work on apogamy, grew prothallia from spores. Such prothallia, when kept dry, in direct sunlight, and watered only from below, developed leaves, roots, and ramenta on the prothallia themselves or on the cylindrical processes. The process continued as a leaf, or it produced sporangia, and tracheids were found both in the process and in other parts of the prothallium. When sporangia were found on a cylindrical process, tracheids were always present in the underlying tissue. YAMANOUCHI (**7**) reports very slow growth and few abortive archegonia in his material kept in bright light and dry air.

My material was not in bright light and was given plenty of moisture, being kept in as nearly normal condition as possible, except for the entire absence of liquid water. Consequently, if this is apogamy, the lack of fertilization is apparently the only factor involved in its appearance here, for in this material archegonia were very numerous and were normal in every respect, and fertilization did take place when water was supplied. In this respect it seems to be like *Marsilea*, where SHAW (**3**) and STRASBURGER (**5**) found that if megaspores of *Marsilea Drummondii* were isolated, and therefore fertilization prevented, parthenogenetic (apogamous) embryos were produced.

The gametophyte number of chromosomes is approximately 16. This number is very suggestive of Osmundaceae (SMITH **4**, STRASBURGER **6**, YAMANOUCHI **8**), which is reported to have 16 chromosomes in the spore mother cell. However, the young sporophyte does not resemble the mature form of *Osmunda*, and whether it resembles the sporeling I cannot tell, not being familiar with the sporelings of *Osmunda*.

The sporophyte structures afforded no opportunity for determining whether it contained the haploid or diploid number of chromosomes. Yamanouchi found the haploid number of chromosomes in apogamous embryos, and concluded that the number of chromosomes is not the only factor which determines the characters of the sporophyte and gametophyte. Strasburger (5) found two kinds of megaspores in *Marsilea Drummondii*, some with the haploid and some with the diploid number of chromosomes. It would be expected that those with the diploid number of chromosomes would develop sporophytes without fertilization, as the gametophyte, and consequently the egg, has the diploid number already present. Farmer and Digby (1) found a vegetative fusion of nuclei in two forms, thus getting the sporophytic number of chromosomes without ordinary fertilization.

The question of apogamy and the literature on the subject will not be discussed further at present, as it is hoped the material may furnish further evidence of this condition.

It is a pleasure to express my obligations to Dr. Charles J. Chamberlain for advice and criticism during this work.

Summary

Prothallia kept for three years in the laboratory in as nearly normal conditions as possible, except for the absence of liquid water, continue to grow, but develop peculiar forms and branching of various types.

The sex organs continue to develop, antheridia being found occasionally on the main plant in all positions, but especially on the branches. Archegonia become very numerous, approximately 300 having been found on one gametophyte. These not only develop in the apical region, but also far back among the old archegonia.

Fertilization may take place whenever liquid water is present, as shown in several cases where gametophytes were placed in water and sectioned later.

Apogamy is present in a sporangium-like structure which lacked the long stalk of the Polypodiaceae, but was not unlike the younger stages of the sporangium of the Osmundaceae. It had two layers of cells outside the fertile region, the inner of these layers resembling a tapetum.

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